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Original research paper

Study of the effect of formulation variables on the characteristics of combination tablets containing enalapril maleate and indapamide as active substances using experimental design

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To evaluate the influence of different variables on tablet formulations containing enalapril maleate and indapamide as active substances, two separate experimental designs were employed: one for evaluating powder properties and the other for tablet characteristics. Because of the low active pharmaceutical ingredient content, it was hypothesized that both powder and tablet properties could be determined only by the characteristics of excipients. In order to test this assumption, both experimental designs were done with placebo mixtures. The optimized formulation was then evaluated both with and without APIs. Results indicated that filler and lubricant percentage, along with compression force, were the most important variables during the formulation study. The optimized formulation showed similar characteristics in both cases for all responses, except for angle of repose and friability where only minor differences were observed. The combination of the applied approaches (using placebo composition and fractional experimental design) proved to be efficient, cost effective and time saving.

Keywords: design of experiments, optimization, factorial design, placebo formulation

Proper management of hypertension, one of the leading causes of cardiovascular disease, is of the utmost importance. It has been established that lowering of blood pressure can effectively reduce the risk of cardiovascular events and stroke, but finding the most appropriate therapy is often difficult (1). Combinational therapies employing an angiotensin-converting enzyme inhibitor (ACEi) and indapamide (IND) offer the advantage of using two highly effective agents, increasing both clinical efficiency and patient compliance (2–4). Low dose combinations of ACEis and diuretics have shown greater reduction in blood pressure and higher success rates in managing hypertension than either of the treatments alone (4–6). The most popular ACEis present in these combinations include perin-

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dopril (2) and delapril (7), but the effectiveness of enalapril maleate (ENA) in combination with IND was also established (8). In some countries, the abovementioned combination is marketed as a commercial product (Enzix Duo), however only as two separate tablets (one containing enalapril maleate and the other indapamide) in the same packaging.

ENA is a relatively inactive pro-drug of the diacid long acting ACEi, enalaprilat. It is widely used in the treatment of essential hypertension, congestive heart failure and several other cardiovascular diseases, such as left ventricular dysfunction (9). IND is a member of the thiazide-like diuretic class, with uses which include hypertension, edema, including that associated with heart failure (9).

In the early drug formulation phase, it is rather challenging to find the optimal composition of the dosage form and the most suitable manufacturing process variables. Traditional formulation development is based on changing one variable at a time, while keeping other variables constant until no further improvement is achieved. This is the so-called COST (changing one separate factor at a time) or OFAT (one factor at a time) method (10). Unfortunately, this method leads to a multitude of experiments, whilst gaining only little information about the process itself. It does not allow identification of the real optimum, because in most cases different starting points will lead to different optimums (10, 11).

In contrast to the COST method, the design of experiments (DoE) relies on a carefully selected representative set of experiments and statistical data processing in order to find optimal conditions. The main advantage of this approach is that it makes it possible to obtain maximum process information with a minimal number of experiments. The selected factors are varied at different levels simultaneously, making it possible to detect not only the statistically significant main effects, but also possible interactions. As a result of DoE, process responses can be described as a mathematical function of several factors, making it possible to predict the outcome of unperformed experiments (12).

Before any experiments are made, some input conditions have to be specified, including the experimental objective, the factors and their range and responses that will be monitored. After these conditions are selected, the experimental design is created and the specified experiments are carried out. The experimental data gained will then be investigated using regression analysis and a model will be created based on the results, indicating which of the selected factors are important and how they influence the selected responses.

One of our main objectives was to systematically investigate the effect of selected process variables on the characteristics of both the powder blend and dosage form. In general, in the phase of early formulation development, a screening phase is desirable, employing a two-level full factorial design (11), assigning a high and a low level for each factor. However, when the number of factors is high, the number of experiments that should be performed increases (for 5 factors, in our case, the number of $2^5 = 32$ experiments would be necessary). Fractional factorial designs are suitable for reducing the number of design runs, while still being able to identify the main effects. In our case, two separate designs were employed.

To characterize powder blends, the proportion of three different excipients and a single process variable was studied by a fractional factorial design of resolution IV and a total of 11 runs ($2^{4-1} = 2^3 = 8$ factorial points and three replicated center points). To investigate tablet properties, a fractional factorial design of resolution III was applied. Apart from the

four variables employed in the previous design, another process variable (compression force) was also studied. A total of 11 runs were employed ($2^{5-2} = 2^3 = 8$ factorial points and three replicated center points).

Besides understanding the effect of variables upon powder and tablet characteristics, optimization of the formulation was also targeted. In order to improve both dosage form aesthetics and for stability reasons, a film coating was applied. Dissolution profiles were evaluated to observe possible release kinetic changes induced by the application of a coating layer.

EXPERIMENTAL

Materials

ENA (Esteve Quemica, Spain) and IND (Bioindustria, Italy) were kind gifts from the local pharmaceutical manufacturing companies. Lactose monohydrate (Flowlac 100) was purchased from Meggle Gmbh (Germany), microcrystalline cellulose (MCC) (Vivapur 102) was obtained from JRS Pharma (Germany), sodium croscarmellose (Disolcel) was from Mingtai Chemical Co. (Taiwan), while colloidal silicium dioxide (Aerosil 200) was purchased from Degussa AG (Germany). Magnesium stearate (MgSt) was purchased from FACI SpA-Carasco GE (Italy) and Opadry II white 85F18422 from Colorcon Ltd. (United Kingdom).

Preparation of powder mixtures and tablets

Prior to mixing, the active substances (if present) and excipients (except MgSt) were passed through a 1 mm hand sieve. The powder blend was then mixed using an Erweka AR 402 mixer (Erweka, Germany) for 3 minutes at a rotation speed of 50 rpm. Thereafter, MgSt was added and further mixed at the same speed for the time specified in the experimental design.

Evaluation of powder mixtures

Flow rate and angle of repose. – In order to evaluate flow behavior of the powder mixture, a total quantity of 100 g was collected from three different sampling points and analyzed using an automated powder testing system (Pharmatest PTG-S4, Pharmatest, Germany). The instrument is suitable for measuring the flow rate and angle of repose in accordance with pharmacopoeial stipulations. A conical stainless funnel, equipped with a pouring nozzle takes the sample to be tested and an analytical balance cell holds the powder collecting dish. Flow rate is measured by recording the time needed for 100 g powder to flow through the pouring nozzle, while the angle of repose of the collected powder mound is also registered. Bulk and tapped density was determined using a Pharmatest PT-TD200 (Pharmatest, Germany), according to method I in both cases, as described in the United States Pharmacopoeia (USP) (14).

Moisture content. – Moisture content of the mixtures was determined with a Halogen moisture analyzer Mettler-Toledo HR 83. The instrument determines the initial mass of the sample and afterwards the sample is heated and the mass loss is monitored until a con-

stant mass is achieved. Once the drying has been completed, the moisture content of the sample is displayed.

Compression of tablets

Tablets containing ENA 10 mg and IND 2.5 mg were prepared employing the direct compression method. Taking into account the quantity of active substances, the tablet mass of 200 mg was selected. Powder blends were compressed into tablets using a Piccola D8 rotating tablet press (Riva, Argentina), at 20 rpm, on 8 mm diameter punches, to a round biconvex form.

Film coating procedure

Opadry II white 85F18422 was selected as film coating agent, a PVA-based fully formulated dry blend for aqueous film coating of immediate release products. The Opadry suspension with 15 % solid content was applied onto the surface of tablet cores at a spray rate of 5 g min^{-1} in a Uni-Glatt type fluid-bed system with Würster insert. Proper tablet surface covering was assured by the selected theoretical mass gain of 3 %. During the whole spraying process, the product bed temperature was maintained between 42 and 44 °C.

Evaluation of tablets

Disintegration time. – Disintegration time was measured according to the method described in European Pharmacopoeia 8 (*Ph. Eur.* 8) (13) using a fully automated six station disintegration tester (Pharmatest PTZ Auto 1EZ, Pharmatest, Germany). Temperature was maintained at 37 ± 0.5 °C and the time taken for each of the 6 tablets to disintegrate was recorded and the average disintegration time was calculated.

Friability. – Friability of tablets was measured according to Ph. Eur. 8, using a Pharmatest PTF 10ER apparatus (Pharmatest, Germany), operated at 25 rpm for 4 minutes. Friability was calculated using the total weight of tablets before and after the test.

Resistance to crushing. – Resistance to crushing was determined with a Pharmatest WHT 3ME apparatus (Pharmatest, Germany) using 10 tablets. The average was calculated from individual values.

Dissolution studies. – Dissolution studies were performed on a Hanson Research SR8 Plus dissolution tester (Hanson Research, USA) using the rotating paddle method (Apparatus 2) at 100 rpm in 900 mL 0.05 mol L^{-1} phosphate buffer pH 6.8, maintained at 37 ± 0.5 °C. Samples of 5 mL were withdrawn at 5, 10, 15, 30 and 45 minutes (for ENA, the last time point was at 30 min) employing an autosampler (Hanson Resarch Autoplus Maximizer and Autoplus Multifill). Samples were separately analyzed for ENA and IND contents according to the HPLC methods described in the United States Pharmacopoeia (USP) monographs Enalapril maleate tablets and Indapamide tablets (14) using an Agilent 1200 DAD-FL modular HPLC system (Agilent Technologies, USA).

Experimental design and statistical analysis

Experimental design and statistical analysis were performed using the Modde 10.1 software (Umetrics, Sweden). Two separate designs were employed: one for the evaluation

of powder mixture characteristics and a separate one for the investigation of tablet properties. The second design was essential for testing the impact of another factor, compression force, on tablet quality. To observe the effect on product characteristics, variables were altered in a larger region. High and low levels for the independent variables were selected according to literature data (15, 16) and our knowledge gained from previous experiments (Table I). Spray-dried lactose content was varied in order to achieve the indicated excipient proportions according to the selected design run.

Table I. Symbols and levels of independent variables used in the design

Variable	Symbol	Levels	
		Low	High
MCC proportion (%)	X1	30	70
Sodium croscarmellose proportion (%)	X2	1	8
Magnesium stearate proportion (%)	X3	0.25	3
Blending time with lubricant (min)	X4	2	10
Compression force (kN)	X5	3	10

For the evaluation of powder mixtures, the dependent variables (responses) were as follows: flow rate (Y1), angle of repose (Y2), moisture content (Y3), bulk density (Y4) and tapped density (Y5). Tablets were characterized in terms of resistance to crushing (Y1′), friability (Y2′) and disintegration time (Y3′).

For the purpose of screening, a linear model, fractional factorial design of resolution IV, was selected to evaluate powder properties (4 factors at 2 levels; 8 factorial and 3 replicate points, totaling 11 runs). Tablets were evaluated according to a linear model, fractional factorial design of resolution III (5 factors, 2 levels; 8 factorial and 3 replicate points, totaling 11 runs). To avoid systematic errors, experiments were randomized and performed according to the generated design.

Obtained models were evaluated using the multiple linear regression (MLR) method. The performance indicators monitored were R^2 (goodness of fit), Q^2 (goodness of prediction), model validity (based on the lack of fit test, as part of the ANOVA evaluation) and reproducibility.

RESULTS AND DISCUSSION

In the pharmaceutical industry, approaches that might shorten drug development timeline are much sought-after. In order to bring clinical or generic candidates through the pipeline, reduction of early formulation development time and costs is crucial. One approach to accomplish this goal is to use the experimental design approach. In our study, the use of two fractional experimental designs was employed in order to gain maximal process information from minimal experimental runs. Since API costs are high, its supply is often limited and, in our formulation, only low dose API was utilized, design runs were performed with placebo mixtures, further minimalizing development costs. To verify the

predicted optimal formulation and its suitability as a dosage form, optimized formulations with and without API were prepared and compared.

Models for powder properties

After obtaining a raw dataset of powder blend analysis (Table IIa), the obtained model was evaluated using the DoE software. The first step in analyzing the data is to evaluate

Table II. Raw data of the experimental design

a) Model for powder properties

Experiment code	Run order	X1 (%)	X2 (%)	X3 (%)	X4 (min)	Y1 (s)	Y2 (°)	Y3 (%)	Y4 (g mL^{-1})	Y5 (g mL^{-1})
N1	8	30	1	0.25	2	35.6	34.2	1.28	0.510	0.625
N2	11	70	1	0.25	10	42.5	36.7	2.42	0.438	0.562
N3	9	30	8	0.25	10	38.1	33.8	1.36	0.541	0.662
N4	3	70	8	0.25	2	52.5	39.0	2.56	0.415	0.556
N5	2	30	1	3	10	36.8	38.9	1.20	0.500	0.625
N6	7	70	1	3	2	58.0	41.3	2.50	0.403	0.529
N7	4	30	8	3	2	40.3	38.9	1.40	0.499	0.610
N8	6	70	8	3	10	54.4	42.5	2.62	0.399	0.532
N9	5	50	4.5	1.625	6	43.1	39.0	1.82	0.442	0.575
N10	1	50	4.5	1.625	6	42.7	37.9	1.90	0.465	0.575
N11	10	50	4.5	1.625	6	48.9	38.7	1.84	0.450	0.581

Y1 – flow rate, Y2 – angle of repose, Y3 – moisture content, Y4 – bulk density, Y5 – tapped density

b) Model for tablet properties

Experiment code	Run order	X1 (%)	X2 (%)	X3 (%)	X4 (min)	X5 (kN)	Y1′ (N)	Y2′ (%)	Y3′ (s)
N1′	3	30	1	0.25	10	10	104	0.08	13
N2′	5	70	1	0.25	2	3	40	0.15	14
N3′	6	30	8	0.25	10	3	11	1.43	16
N4	2	70	8	0.25	2	10	167	0.02	45
N5	9	30	1	3	2	10	83	0.20	34
N6	8	70	1	3	10	3	28	0.43	18
N7	7	30	8	3	2	3	7	3.32	31
N8	10	70	8	3	10	10	117	0.07	97
N9	11	50	4.5	1.625	6	6.5	69	0.08	24
N10	1	50	4.5	1.625	6	6.5	69	0.12	23
N11	4	50	4.5	1.625	6	6.5	65	0.17	28

Y1′ – resistance to crushing, Y2′ – friability, Y3′ – disintegration time

the replicate plot for each response (Fig. 1a). In the replicate plot, response values are plotted against experimental run numbers, replicate runs being shown on the same bar (11). In all cases, variations of replicated center points were much smaller than the variation in the entire investigation series, meaning that replicate errors did not interfere with data analysis.

In regression analysis, normally distributed responses give better model estimates and higher reliability (11). Histogram plots of responses indicated approximately normal distributions (results not shown) for all dependent variables, except for the flow rate, for

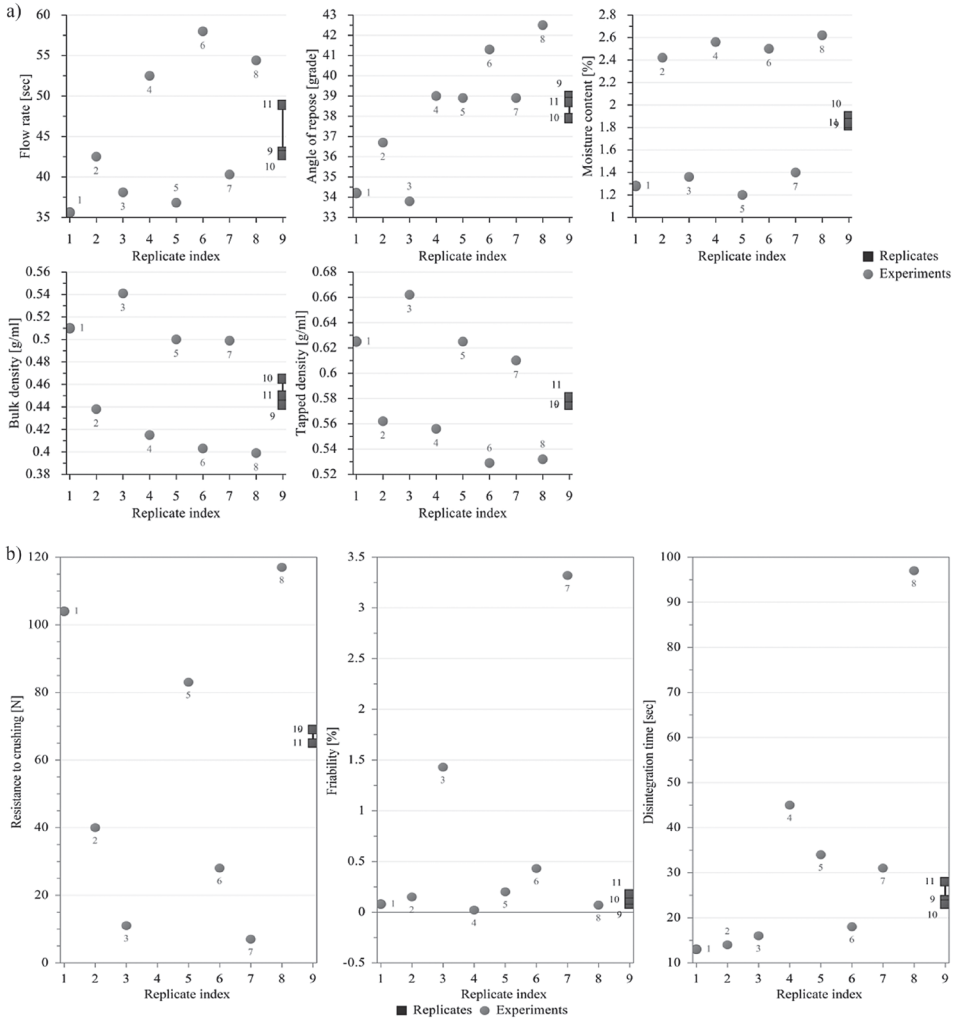

Fig. 1. Replicate plot of responses: a) model for powder properties, b) model for tablet properties. Numbers represent individual experiments.

which the distribution of results showed positive skewness. A simple logarithmic transformation was applied to normalize the distribution for the dataset.

The Summary of Fit Plot for all responses is shown in Fig. 2a. For a model to pass this diagnostic test, both R^2 and Q^2 should have high values, while the difference between the two terms should not be more than 0.2–0.3, higher values indicating an inappropriately selected model (11).

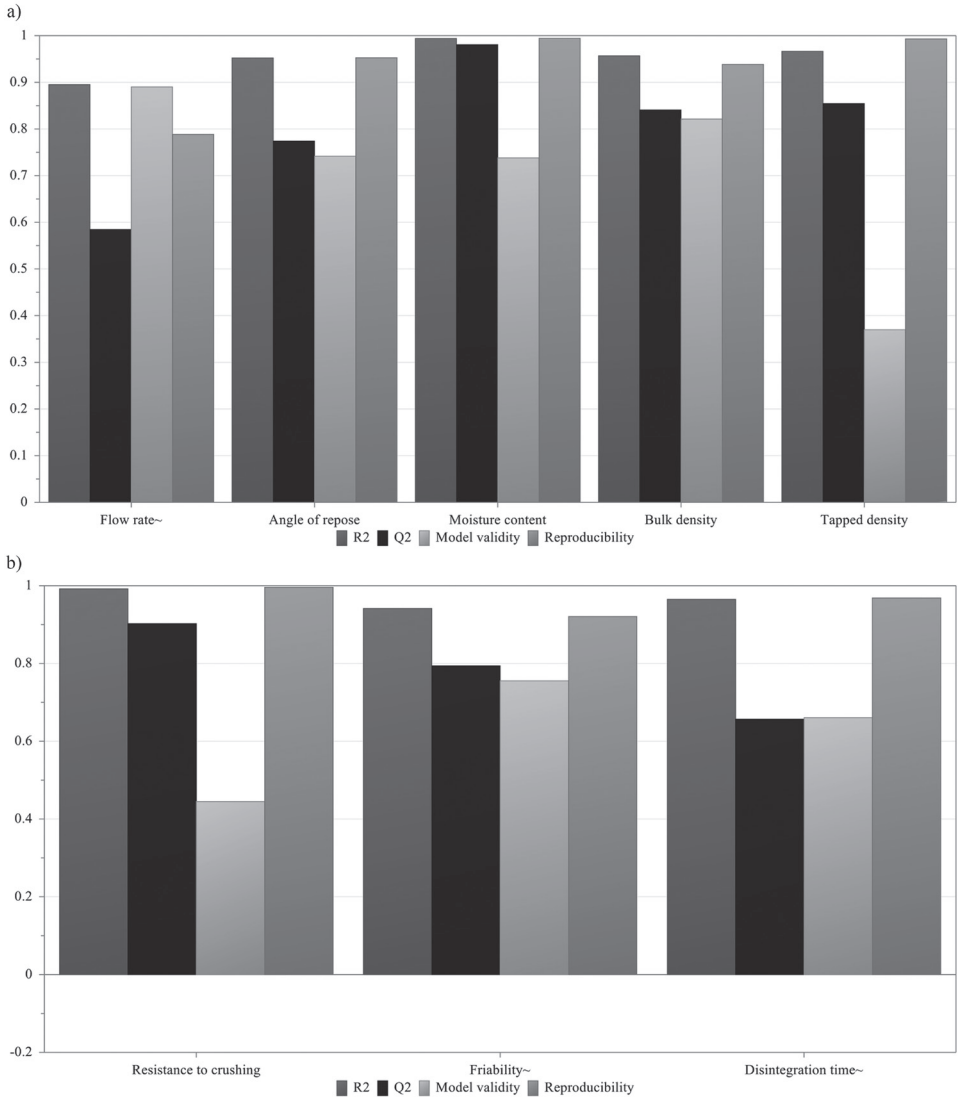

Fig. 2. Summary of fit plot: a) model for powder properties, b) model for tablet properties.

The linear models selected showed good quality, with R^2 values greater or equal to 0.90 and Q^2 values ranging from 0.58 (Y1, flow rate) to 0.98 (Y3, moisture content). Model validity (third bar) shows whether the appropriate model was selected. Values greater than 0.25 point to a valid model, while lower values indicate possible model selection problems. For the models selected, all values were greater than or equal to 0.37, while in the case of reproducibility (fourth bar), values greater than 0.79 were achieved. In general, reproducibility values lower than 0.5 indicate poor experimental control and large pure error (11).

Another important diagnostic test of model validity is the analysis of variance (ANOVA) and its lack of fit test. Table IIIa represents the extracted summaries of the tests for all responses. Two F-tests were performed during the analysis; the first assessed the significance of the response model, while the second test compared model error and replicate error (11). For all responses, significant regression models were obtained ($p < 0.05$) and no lack of fit was detected ($p > 0.05$).

Models for tablet properties

Tablets were prepared according to the selected design and evaluated for the specified responses. Raw data of the results is presented in Table IIb. The fitted model passed the replicative plot analysis for all responses, pointing to low replicate errors (Fig. 1b).

Normal distribution plot of the data was obtained for resistance to crushing, but not for friability and disintegration time. In both cases, the histograms showed strong positive

Table III. Extracted summaries of ANOVA evaluation for the responses

Model for powder characteristics

Response	Regression		Lack of fit	
	F-value	*p*-value	F-value	*p*-value
Y1	12.833	0.004	0.737	0.645
Y2	29.810	< 0.001	2.029	0.356
Y3	252.186	< 0.001	2.067	0.352
Y4	33.259	< 0.001	1.250	0.490
Y5	42.995	< 0.001	11.639	0.081

Y1 – flow rate (s), Y2 – angle of repose (°), Y3 – moisture content (%), Y4 – bulk density (g mL^{-1}), Y5 – tapped density (g mL^{-1})

Model for tablet characteristics

Response	Regression		Lack of fit	
	F-value	*p*-value	F-value	*p*-value
Y1′	101.612	< 0.001	8.176	0.109
Y2′	16.055	0.004	1.801	0.376
Y3′	27.620	0.001	3.029	0.258

Y1′ – resistance to crushing (N), Y2′ – friability (%), Y3′ – disintegration time (s)

skewness, indicating that the frequency of lower values was much higher. In order to achieve approximately normal distribution plots, logarithmic transformations were used for both responses. These transformations resulted in an increase of Q^2 from 0.13 to 0.86 (Y2′ friability) and 0.66 from –0.40 (Y3′ disintegration time). For Y1′ (resistance to crushing), the value obtained for experiment N4′ (167 N) was detected as an outlier and was eliminated, resulting in better model performances.

The Summary of Fit plot (Fig. 2b) indicated that the linear model had acceptable quality, with R^2 values between 0.94 and 0.99 and Q^2 values between 0.66 and 0.94; the highest difference between the two indicators was 0.31 (Y3′ disintegration time). The models were characterized by high reproducibility, while model validities were within acceptable limits, the findings being supported by the good performances during ANOVA analysis (Table IIIb).

Evaluation of variables affecting powder mixture properties

Flowability of powder mixtures has a great influence on tableting characteristics, since it confers a uniform feed of the powder into the tablet press, ensuring mass and content uniformity (17, 18). To study the influence of the variables on flow behavior, coefficient plots were constructed using the DoE software (Fig. 3a). Among the studied variables, MCC proportion (X1) proved to have the greatest impact on powder characteristics, significantly influencing all responses. Cellulose type was shown to have a great influence on the resulting tablet properties (19–21). Vivapur 102 is a fine-grade microcrystalline cellulose and due to its anisometric particles and low density, its mass flow is lower compared to other diluents (22). Accordingly, in our case, powders with increased cellulose content also showed poorer flowability compared to formulations with higher lactose content. Flowlac 100, characterized by its spherical shape of spray-dried aggregates, larger particle size and thus low cohesion (23) confers better flow characteristics to powder blends.

Apart from the MCC content, the flow rate also decreased with increased lubricant percentage (X3); however, the negative effect on powder flow was more pronounced when measuring the angle of repose. This behavior came as a surprise, since increased magnesium stearate content of powders was linked with improved flow characteristics (24–26) by reducing adhesion and capillary forces (27, 28). Lubrication, however, is not only dependent on the lubricant content, but also on the blending time and mixing intensity. In particular, when mixed for longer periods, several lubricants formed films on the surface of larger particles, which in turn improved flowability (29–31). Examining the anomaly in our situation, it is presumed that the interval of mixing time with lubricant could have led to inadequate blending. This assumption is based on the observation that mixing time had a slight positive effect on both the flow rate and angle of repose (*i.e.*, small decrease in both parameters with prolonged mixing time). Analyzing the moisture content of the powder blends, MCC proportion showed a strong positive correlation, as expected, while other variables had only a small or no effect at all. Higher moisture content of powder blends with increased MCC proportion is thoroughly discussed in literature, in fact, hygroscopic behavior of MCC is one of its main limitations when used as a diluent (32–35).

Densities of the prepared powder mixtures decreased with increased MCC content, mostly due to an increase in cohesive forces between MCC particles when compared to spray dried lactose.

Evaluation of variables affecting tablet properties

Tablets were evaluated in terms of resistance to crushing (Y1′), friability (Y2′) and disintegration time (Y3′). The coefficient plot for the selected responses (Fig. 3b) revealed the significance of compression force (X5) upon tablet characteristics, which, in our case, proved to be the most important factor to control. As expected, there was a strong positive

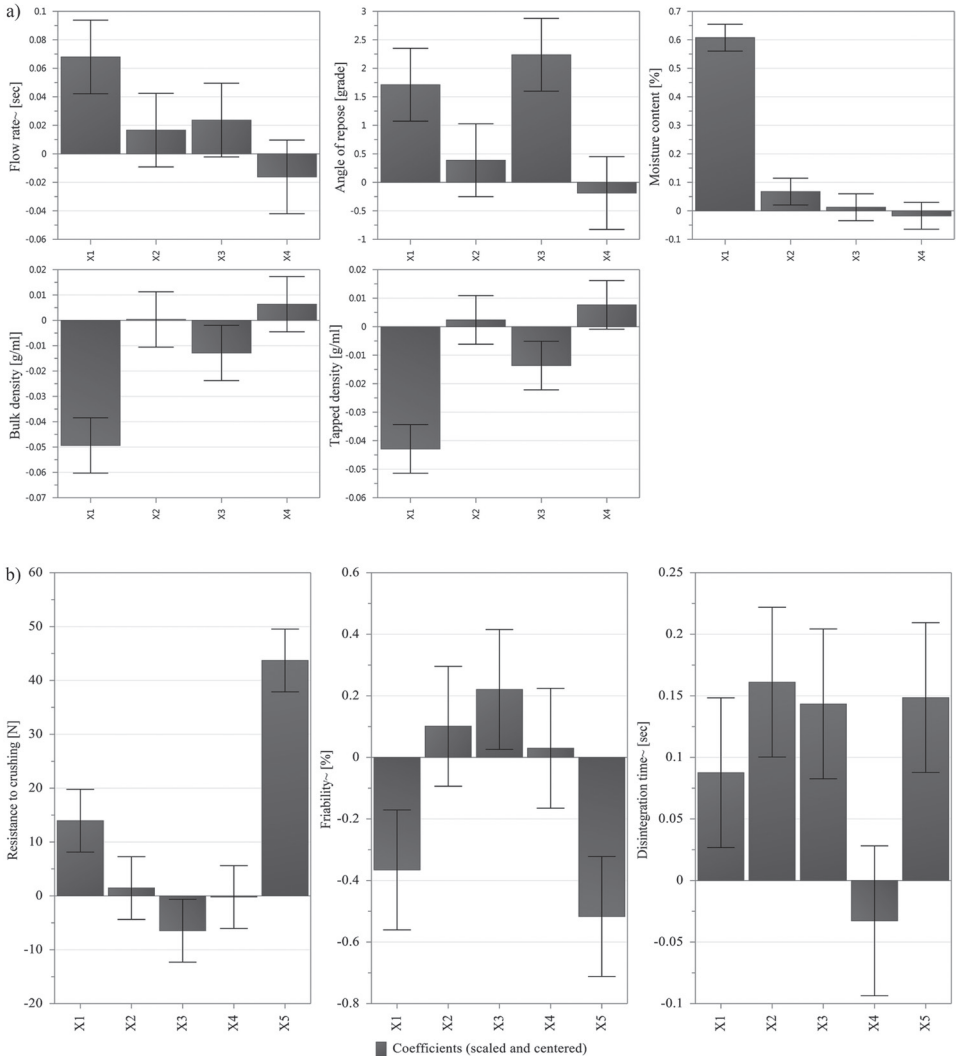

Fig. 3. Coefficient plots for responses: a) model for powder properties, b) model for tablet properties. X1 – MCC proportion (%), X2 – sodium croscarmellose proportion (%), X3 – MgSt proportion (%), X4 – bending time with lubricant (min), X5 – compression force (kN).

linear correlation between compression force and resistance to crushing (correlation coefficient, $R = 0.94$). Except for experiment N4′ (resistance to crushing 167 N), values obtained for this parameter were between 7 and 117 N, with center points situated around the value of 70 N. Although in a smaller proportion than compression force, MCC content (X1) also proved to be significant for achieving high tablet hardness. Increasing the MCC ratio resulted in an increase in resistance to crushing, cellulose exhibiting better compactibility and thus conferring higher tablet hardness (36). Increasing the MgSt content (X3) led to a small decrease in resistance to crushing values, while other factors did not seem to contribute significantly.

In the case of uncoated tablets, friability not greater than 1 % is considered acceptable under current regulations. All formulations, except for N3′ and N7′, showed lower friability values than the maximum allowed ones. It is noteworthy that the two mentioned formulations exhibited also the lowest resistance to crushing (11 N and 7 N, respectively). Upon examination, the correlation matrix revealed a negative correlation between resistance to crushing and friability, higher tablet hardness being related to lower friability values ($R = –0.86$). This finding was also supported by the lower friability values obtained when employing higher compression forces. Increase in lubricant content (X3) led to an increase in friability; however, the impact of the variable was lower than that of the other two. Sodium croscarmellose content (X2) and lubrication time (X4) seemed to have no significant effect on either of the two responses (resistance to crushing and friability).

When formulating immediate release tablets, rapid disintegration is considered in order to rapidly release the active substance from the formulation and make it readily available. For uncoated tablets, unless stated otherwise, current pharmacopoeial regulations stipulate a maximum disintegration time of 15 minutes. As the raw dataset demonstrates (Table IIb), all formulations disintegrated in less than 2 minutes, with the highest values obtained for N8′ (97 s).

Increasing the compression force reduces liquid penetration into tablets, which in turn increases disintegration time (37), the behavior that was observed also in our case. Disintegration time also increased with lubricant proportion, probably due to the hydrophobic film forming capacity of magnesium stearate (38) and it also increased with the MCC content.

Croscarmellose sodium has a high swelling capacity even at low concentrations, disintegrating tablets rapidly (37, 39), which in theory should have a positive effect on disintegration time (*i.e.,* decrease with increased concentration); however, in our particular case, it increased disintegration time. A possible explanation might be that sodium croscarmellose reached its peak efficiency at low concentrations and higher ratios of the disintegrant with fibrous structure led to a slight increase in disintegration time due to cohesive forces. Also, the recorded resistances to crushing values were on the lower side, facilitating water penetration into tablets and allowing even low concentrations of the disintegrant to swell rapidly.

Formulation optimization

After completion of the screening phase, the formulation was optimized using the Optimizer feature of the DoE software**,** applying the following criteria: target value for resistance to crushing 80 N (min. 60 N, max. 100 N), friability minimized, with a target

Table IV. Predicted and practically obtained response values for the optimized formula[a]

Predicted values						Y1′ (N)	Y2′ (%)	Y3′ (s)
						80	0.18	23
Results obtained	Y1 (s)	Y2 (°)	Y3 (%)	Y4 (g mL^{-1})	Y5 (g mL^{-1})	Y1′ (N)	Y2′ (%)	Y3′ (s)
Without APIs	34.6	36.1	1.4	0.497	0.625	76	0.11	19
With APIs	34	39.2	1.48	0.491	0.625	73	0.26	18

[a]Optimized formula: MCC proportion (X1) 30.8 %; sodium croscarmellose proportion (X2) 4.64 %; MgSt proportion (X3) 0.99 %; compression force (X4) 8.5 kN; blending time with lubricant (X5) 6 min.

value of 0.2 % (max. 1 %), disintegration time minimized, with a target value of 60 s (max. 90 s).

To test the impact of the small amount of active pharmaceutical ingredients (APIs) on powder and tablet characteristics, the optimized formulation was prepared both with and without the APIs. Table IV shows the closeness of the predicted and actually obtained results for both formulations. As can be seen, differences were found only in the case of friability, which was still much below the pharmacopoeial limit of 1 %. Similar results were obtained for powder characteristics, where differences were obtained only when measuring the angle of repose of the powder blends (39.2 and 36.1 ° for the formulation with and without APIs, respectively).

In order to obtain a pharmaceutical formulation that could be used in practice, the tablets were coated and *in vitro* dissolution studies were performed for both coated and uncoated tablets. The coating was applied not only for aesthetic, but also for stability considerations. However, since an immediate release of APIs was targeted, the coating should not prolong dissolution. Both formulations demonstrated very similar release profiles, indicating that the application of a film layer did not change the release rate significantly (Fig. 4). Moreover, dissolution of both APIs corresponded with the USP stipulations (NLT 80 % dissolved in 30 minutes for ENA and NLT 75 % dissolved in 45 minutes for IND), making the present formulation an interesting and cheaper alternative to other combination therapies present on the market. However, further studies need to be performed both in preclinical and clinical phases in order to test the stability, efficacy and suitability of this combination.

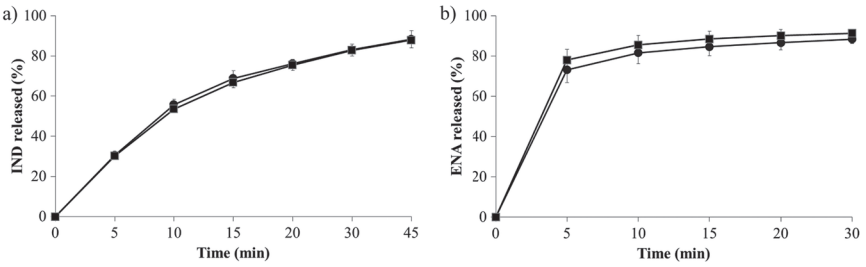

Fig. 4. Comparative dissolution profiles of both coated (■) and uncoated (●) formulations (n = 6) for: a) ENA and b) IND. Error bars represent standard deviation values.

CONCLUSIONS

The combination of two widely used and highly efficient antihypertensive agents, ENA and IND, was used in a pharmaceutical formulation as a possible cheaper alternative to currently used antihypertensive combinations. Design and evaluation of the formulation were performed with the aid of DoE software in order to observe the effects of different formulation and process related variables on the selected responses. To evaluate both powder and tablet properties, two separate experimental designs were constructed using the software. Since the amount of APIs in the formulations was low, it was hypothesized that both powder and tablet properties would be determined only by the characteristics of excipients. To test this assumption, both experimental designs were applied with placebo composition (without API). Analyzing the results of experimental runs, it was concluded that MCC and MgSt contents were the most important factors affecting powder characteristics. In terms of tablet characteristics, compression force proved to be the most important factor to control, followed by MCC and lubricant contents.

Based on the available data, the formulation was optimized using the DoE software. Optimized formulations were prepared both with and without APIs. Obtained results were in good accord with the predicted values and no differences were found between the formulations with and without the APIs for the majority of responses.

In the final phase, tablets were coated for both aesthetical and stability reasons. Dissolution studies were performed and both coated and uncoated tablets met the pharmacopoeial requirements for both APIs, indicating that the coating did not interfere with the immediate release of active substances. The developed formulation could be a promising cheaper alternative to current combination treatments for hypertension. Moreover, the combined approach of using placebo mixtures and fractional factorial designs proved to be highly efficient, leading cost-effectively to optimal composition in a time saving manner.

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